Occurrence and conservation of the threatened endemic cobitid, *Cobitis calderoni,* in the Erro River (Ebro Basin, Spain)

by

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ABSTRACT. - The present study contributes to the knowledge about the distribution of the scattered and scarce populations of the threatened Iberian endemic fish species, lamprehuela (*Cobitis calderoni* Bacescu, 1961) within the Ebro River basin. The Erro River was thoroughly surveyed to: 1) identify and describe reaches with suitable habitat, and 2) to elaborate a detailed distribution map in order to give conservation recommendations for the species within this protected area. The length-weight relationship ($a = 0.0018$, $b = 3.3972$, $r^2 = 0.9483$, $p < 0.0001$) as well as mean Fulton' condition factor (K = 0.3477, SE = 0.0083) for *C. calderoni*, and its occurrence in the Irati and Ega Rivers (Ebro River basin) are reported here for the first time.

RÉSUMÉ. - Répartition et protection de l'espèce endémique menacée *Cobitis calderoni* dans l'Erro, un tributaire du bassin de l'Ebre, Espagne.

L'objet de cette étude est de compléter nos connaissances sur la distribution très clairsemée des populations d'un poisson endémique rare et menacé dans le bassin de l'Ebre, la loche de Calderon (*Cobitis calderoni* Bacescu, 1961). La rivière Erro a été soigneusement prospectée pour identifier les secteurs convenables du point de vue de l'habitat, et établir une carte de répartition détaillée permettant d'émettre des recommandations pour la conservation de cette espèce dans cette zone protégée. L'étude a permis d'établir pour la première fois la relation longueur-poids chez cette espèce ($a = 0{,}0018$; $b = 3{,}3972$; $r^2 = 0{,}9483$; $p < 0{,}0001$), de calculer la moyenne du facteur de condition de Fulton ($K = 0{,}3477$, SE = 0,0083) et de découvrir sa présence dans les rivières Irati et Ega (bassin de l'Ebre).

Key words. - Cobitidae - *Cobitis calderoni* - Distribution - Conservation - Habitat - Endemism - Lamprehuela.

In order to manage endangered species effectively, it is necessary to identify the reason for decline and that is best approached by field studies on declining populations and a severe understanding of the ecology of the target species. Even though the zoogeographic integrity coefficient (native to total number of species ratio) of the Ebro basin is moderate (0.58; Elvira, 1995a), most of the native species are endemic of the Iberian Peninsula. In the Ebro basin, the degree of endemicism, measured as the ratio between Iberian endemic species to the total number of native species of a given family (Elvira, 1995a), is especially high in Cyprinids (75%), Cyprinodontids (100%) and Cobitids (100%).

The lamprehuela, *Cobitis calderoni* Bacescu 1961, is a small-bodied freshwater fish species that is endemic to the Iberian Peninsula, occurring within the Douro and Ebro basins as well as a few locations in the Tagus basin (Elvira, 1995b; Doadrio, 1981). Known populations of *C. calderoni* within the Ebro River basin are few and scarce (Doadrio, 2001). *C. calderoni* is listed as endangered (EN A2ace+3ce) in the IUCN Red List of Threatened Species (2006), and is included in both the Spanish and Portuguese National Red Lists of Threatened Species (Bohlen and Ráb, 2001). At regional scale, it is catalogued as of "Special Concern" in the wildlife act of the province of Navarre (Orden Foral 0209/1995). In that province, *C. calderoni* was first cited in the Ebro River and the lower reaches of one of its tributaries, the Aragón River (Doadrio, 1981). But recently, its distribution range was extended to the Erro (García-Fresca, 2003; C.H.E., 2005; Miranda *et al.*, 2005), Esca and Salazar Rivers (Sainz de los Terreros, 2002), which are all tributaries of the Aragón River (Fig. 1).

C. calderoni inhabits higher and middle reaches of rivers with clear, well-oxygenated waters (usually higher than 7-8 $mg \cdot l^{-1}$ O_2; Bacescu, 1961; García de Jalón and López Álvarez, 1983) with gravel and rocky bottoms. The species exhibits an unbalanced sex-ratio, with males being very rare (Perdices and Doadrio, 1997). Spawning takes place from March through May, when gravel bottoms with strong water currents are chosen (Bacescu, 1961).

The leading threat causes for *C. calderoni* include the destruction of the spawning areas due to gravel extraction, hydraulic constructions (Miranda *et al.*, 2005), water extraction, water pollution (Doadrio, 2001), and introductions of non-native predatory fishes (Rincón *et al.*, 1990). Doadrio (2001) estimated that the distribution range of the species has been reduced by about 20% during the 1990s, especially within the Ebro basin, where this range reduction could be as much as 50%. The objectives of the present study were to:

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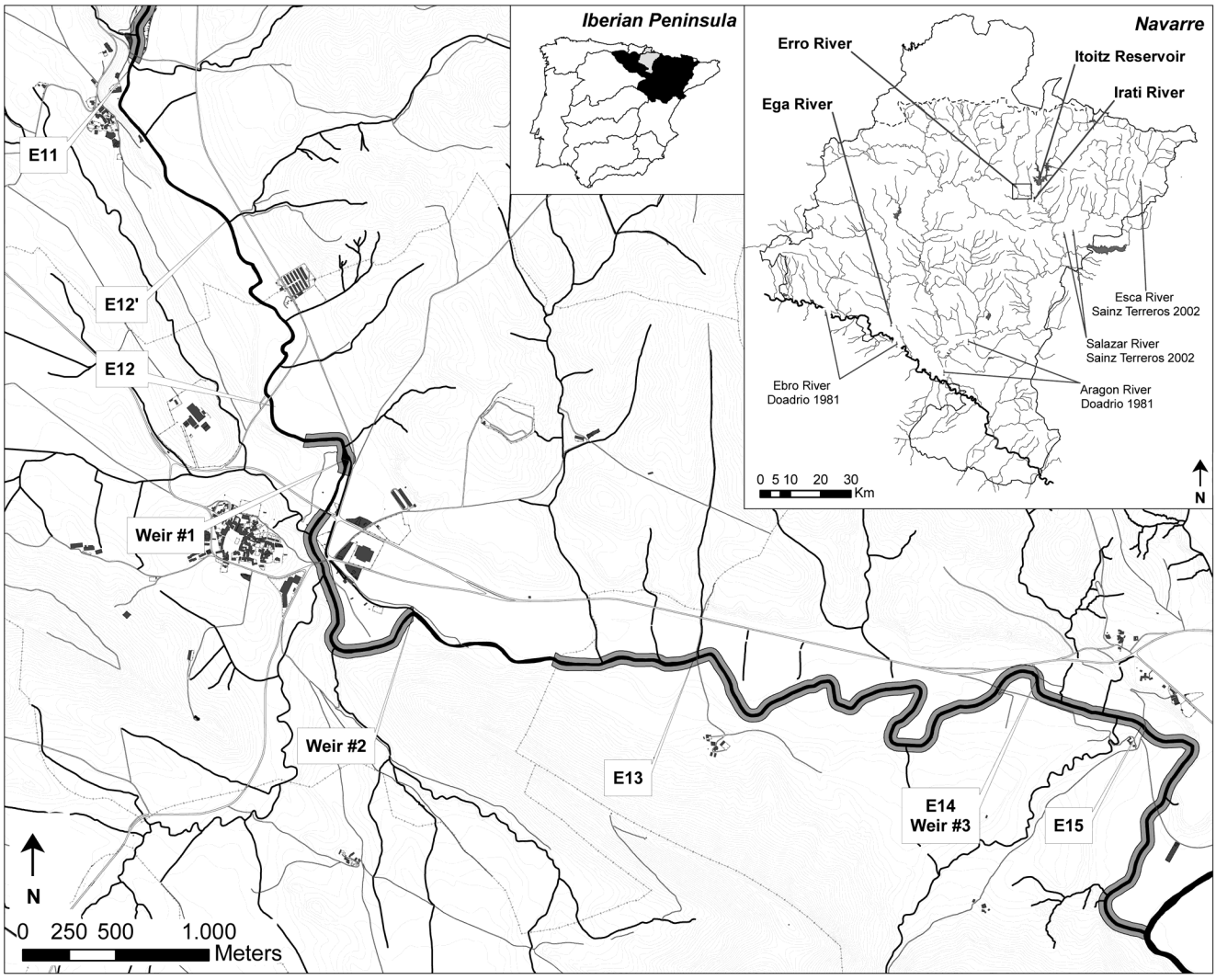

Figure 1. - Map with the location of the sampling sites, weirs and inadequate habitat (parallel shading) for *Cobitis calderoni* Bacescu, 1961 along the lower Erro River. Known geographical distribution of *C. calderoni* within the province of Navarre (right inset), and the location of the Ebro Basin and Navarre in the Iberian Peninsula (left inset). *[Carte de la rivière Erro avec localisation des sites d'échantillonnages, des seuils et des secteurs défavorables (surlignés en grisé) pour* Cobitis calderoni. *Répartition géographique actuellement connue de l'espèce en Navarre (grand encart). Petit encart : la Navarre et le bassin de l'Ebre à l'échelle de la péninsule ibérique.]*

1) corroborate the present occurrence and distribution of *C. calderoni* along the Erro River; 2) identify the main factors that threaten the species; and 3) report on the first recording of the species in the Irati and Ega rivers.

MATERIAL AND METHODS

Sampling was undertaken in tributaries of the Ebro River, which are located within the province of Navarre (Fig. 1), using a back-pack electrofishing unit (ACUITEC - Martín Pescador III, 300-600 V, 0.2-2 A) according to three sampling designs (i.e., three-run depletion between two stop-nets, qualitative surveys, semi-quantitative surveys) depending on the original purpose of the survey. Electrofishing in the Irati River was carried out just downstream the bridge of Aós in summer 2005. The semi-quantitative survey in the Ega River was performed at the locality of Cárcar in summer 2006.

Along the Erro River, fifteen sampling sites (E01 to E15) were chosen, including locations (i.e., E11: Lizoain and E14: Villaveta) where *C. calderoni* was reported previously (García-Fresca, 2003). All fifteen reaches were electrofished quantitatively during the summer 2001, in every season in 2002 and in summer 2005. Additionally, all the wadeable reaches of the Erro River were surveyed in summer 2005

between E11 and E14 (Fig. 1) in order to find suitable habitat and therefore, previously unknown locations for the species. A detailed description of the Erro River can be found elsewhere (García-Fresca, 2003; Leunda *et al.*, 2004; Miranda *et al.*, 2005).

Captured fishes were anaesthetized with 2-phenoxyethanol (0.1-0.2 mg·l^{-1}: Summerfelt and Smith, 1990) before being measured to the nearest 0.1-cm total length (TL). In summer 2005, the fish were also weighted using a digital balance (to 0.01 g) to obtain total body weight (W). All specimens were returned to the water alive, so age and/or sex of the specimens could not be determined. In the laboratory, the length-weight relationship was calculated using the equation $W = a \cdot L^b$, whereby isometric growth takes place when coefficient $b = 3$ and parameters a and b were estimated using linear regressions $\log W = \log a + b \cdot \log L$ on $\log_{10}$ transformed data. Additionally, the mean value of Fulton's condition factor was estimated using the equation $K = (W \cdot L^{-3}) \cdot 10^5$ (Murphy and Willis, 1996).

RESULTS

The electrofishing surveys detected localized and scarce populations of *C. calderoni* just downstream the bridge of Aós in the Irati River in summer 2005 (0.97 fish·100 m^{-2}) and in the locality of Cárcar in the Ega River in summer 2006 (14.40 fish·h^{-1}, CPUE).

During our seasonal surveys in 2001 and 2002 at all fifteen sampling sites along the Erro River (E01 to E15), we found *C. calderoni* at E12 only (Fig. 1), where (in decreasing abundance) European minnow *Phoxinus phoxinus* (Linnaeus, 1758), Iberian nase *Chondrostoma miegii* Steindachner, 1866, Iberian barbel *Barbus graellsii* Steindachner, 1866, gudgeon *Gobio lozanoi* Doadrio & Madeira, 2004, stone loach *Barbatula barbatula* Linnaeus, 1758 and brown trout *Salmo trutta* (Linnaeus, 1758) are also found. The summertime electrofishing surveys in E12 revealed the highest density in 2001 (34.6 fish·100 m^{-2}), lower values in 2002 (13.0 fish·100 m^{-2}) and in 2005 (3.5 fish·100 m^{-2}) (Tab. I).

		Summer Surveys in E12		
Day		20/08/01	13/08/02	2/08/05
Water temperature (ºC)		18.3	17.2	17.4
Conductivity (μS·cm^{-1})		411	403	367
Total dissolved solids (mg·l^{-1})		412	404	414
Dissolved oxygen (mg·l^{-1})		8.26	8.92	7.28
pH		7.78	8.38	7.84
Width	Mean (m)	5.1	6.7	6.0
	Range (m)	(2.2-7.5)	(3.8-11.1)	(3.5-9.9)
Depth	Mean (cm)	42	30	23
	Range (cm)	(13-78)	(6-71)	(6-43)
	Low (%, < 30 cm)	20	58	73
	Moderate (%, 30-60 cm)	60	29	27
	High (%, > 60 cm)	20	13	0
Discharge	Day Mean (m^3·s^{-1})	0.50	0.65	0.06
	Previous 30-day range (m^3·s^{-1})	(0.45-0.75)	(0.50-0.65)	(0.06-0.18)
Water velocity	Low (%, < 0.3 m·s^{-1})	93	60	91
	Moderate (%, 0.3-0.75 m·s^{-1})	7	20	9
	High (%, 0.75-1.2 m·s^{-1})	0	13	0
	Very High (%, > 1.2 m·s^{-1})	0	7	0
Substrate type	Fines (%, < 2 mm)	0	0	0
	Gravels (%, 2-64 mm)	16	19	7
	Pebbles (%, 64-256 mm)	74	81	82
	Boulders (%, > 256 mm)	10	0	11
	Bedrock and concrete	0	0	0
Shade (%)		·	29	64
Surveyed area (m^2)		264	348	249
Cobitis calderoni	< 40 mm (fish·100 m^{-2})	21.2	1.5	0.0
	40-50 mm (fish·100 m^{-2})	2.1	6.5	1.0
	50-60 mm (fish·100 m^{-2})	7.9	3.8	2.5
	> 60 mm (fish·100 m^{-2})	3.3	1.1	0.0
	Total (fish·100 m^{-2})	34.6	13.0	3.5

Table I. - Reach-scale habitat features and estimated densities of *Cobitis calderoni* in the E12 site during the summertime sampling occasions. Discharge data from the flow gauging station at E14. *[Caractéristiques de l'habitat et densités estimées de* Cobitis calderoni *du secteur E12 pendant l'échantillonnage d'été. Les données de débits proviennent de la station de jaugeage située en E14.]*

Additional seasonal surveys in E12 showed low densities in winter 2001-2002 (0.4 fish·100 m^{-2}; i.e. only one specimen), none in spring 2002, but a higher value in autumn 2002 (10.5 fish·100 m^{-2}). Moreover, the additional electrofishing survey carried out in summer 2005 along all the wadeable reaches with suitable habitat for *C. calderoni* revealed another group of three individuals in E12' (Fig. 1). With the length (28-61 mm TL) and weight (0.07-0.95 g) of all the specimens captured in summer 2005 (n = 31) the equation of the length-weight relationship (a = 0.0018, b = 3.3972, r^2 = 0.9483, $p < 0.0001$) as well as mean Fulton's condition factor (K = 0.3477, SE = 0.0083) were computed.

DISCUSSION

During electrofishing surveys in summer 2005 downstream of Itoitz Reservoir, to assess the initial impact of this water retention structure on the Irati River's fish assemblage, a localized and scarce population of *C. calderoni* was detected just downstream the bridge of Aós. Another population of the species was also detected during the semi-quantitative surveys carried out in summer 2006 in the Ega River for the elaboration of a technical report. These constitute the first records for *C. calderoni* in the Irati and Ega Rivers (Fig. 1). A more thorough distribution survey is needed in the Irati River because of the flow regulation/alteration that recently started with the bringing into operation of the Itoitz Reservoir. Such alterations of the water level and current speed are likely to affect the species negatively.

Summertime surveys on the Erro River in 1995 to 1997 (García-Fresca, 2003; C.H.E., 2005) revealed *C. calderoni* populations in the Erro River (Fig. 1), including low densities at locations downstream the weir #2 (C.H.E., 2005), E11 and E14 (García-Fresca, 2003). During our surveys in 2001 and 2002 at sites upstream (E10, E12) and downstream (E13, E15) of E11 and E14, we found *C. calderoni* at E12 only (Fig. 1), where co-occurs with several other endemic (*C. miegii, B. graellsii, G. lozanoi*) and native species (*P. phoxinus, B. barbatula, S. trutta*) constituting a near-natural fish assemblage free of exotic species. The local extirpation of *C. calderoni* at E14 has been attributed to the construction of a compound flow gauging weir (weir #3, Fig. 1) in the reach at E14 (Miranda *et al.*, 2005), suggesting that habitat alteration related to river regulation structures are a major threat for this vulnerable species (Doadrio, 2001).

Even though the additional electrofishing survey carried out in summer 2005 along all the wadeable reaches with suitable habitat for *C. calderoni* revealed another group of three individuals in E12' (Fig. 1), the summertime electrofishing surveys in E12 showed a decrease in density from 2001 to 2005 (Tab. I), whilst the additional seasonal surveys mostly showed lower densities. These additional seasonal estimates may be biased by the seasonal catchability of this bottom-dwelling species, with small body size, mimetic body coloration, cryptic behaviour and lack of a swimbladder influencing catchability through active fishing methodologies (Zalewski and Cowx, 1990; Clavero *et al.*, 2006). We do not believe that the apparent sequential population decline is a consequence of a long-term effect of the electrofishing surveys because in every occasion all the captured specimens were returned alive to the river.

To our knowledge the equation of the length-weight relationship ($W = 0.0018 \cdot TL^{3.3972}$, r^2 = 0.9483, $p < 0.0001$) as well as the mean Fulton's condition factor (K = 0.3477, SE = 0.0083) are described here for the first time for *C. calderoni*. Values of b larger than 3 indicate positive allometric growth (t = 2.6947, df = 30, p = 0.0114), somewhat previously reported for another cobitid species present in the Iberian Peninsula, *C. paludica* (b = 3.541, Bravo *et al.*, 2001; b = 3.5415 Soriguer *et al.*, 2000).

C. calderoni is reported to require clear, fresh and well-oxygenated waters running in gravel/pebble bottoms (Bacescu, 1961; García de Jalón and López Álvarez, 1983; Doadrio, 2001), and this description fits that of reach E12 (Tab. I) (Leunda *et al.*, 2004). Taking into account, on one hand, that the species was reported by Garcia-Fresca (2003) in E11 and in this study in E12', and, on the other hand, the similarity (Morisita, M) in substrate composition (M = 0.9764), depth distribution (M = 0.9964) and hydrochemistry between E11 and E12 (Leunda *et al.*, 2004), we consider that the whole stretch between E11 and E12 (ca. 2.4 km) fulfils the described habitat requirements of *C. calderoni*. On the contrary, several reaches of the lower Erro River are inadequate for *C. calderoni* due to different physical characteristics and reach features. Downstream E12, the river is impounded by a 2.5-m high weir (weir #1, Fig. 1) constructed in 1993 for hydroelectric power production. Similarly, the impoundment affects the river along the Urroz town due to a smaller weir (weir #2, Fig. 1). Moreover, Leunda *et al.* (2004) showed that the riverbed along the reaches E13 to E15 predominantly consists of bedrock, even thought some patches of suitable habitat could be found. Specifically, the dissimilarity in the substrate composition in E13 and E14 reaches against E12 ($M_{E12 \text{ vs } E13}$ = 0.2262, $M_{E12 \text{ vs } E14}$ = 0.3271, $M_{E13 \text{ vs } E14}$ = 0.9526) is basically due to dominance by bedrock (Leunda *et al.*, 2004).

Even thought the described situation is not optimistic, the Erro River offers an opportunity for the conservation of the scarce and localized *C. calderoni* populations. Doadrio (2001) claimed the necessity to protect areas at which the species inhabits within all the three Ebro, Tagus and Douro basins in order to ensure species populations survival. The Erro River was recently included in the Natura 2000 Net-

work, and though little attention was paid to the fish fauna for the designation, the ichthyofauna must be a key element for the elaboration and implementation of the management plan for this protected area. Therefore, following the conservation recommendations for *C. calderoni* (Maitland, 1995; Perdices and Doadrio, 1997; Doadrio, 2001) and learning from previous experiences (Miranda *et al.*, 2005), it is proposed to control (1) the gravel and water extraction, (2) the introduction of invasive exotic fish – specially piscivorous species – and (3) the impact of future hydraulic constructions. Additionally, (4) the dismantling of the weirs #1 and #2 would create more suitable habitat available for *C. calderoni*.

Beyond the local limits of the Erro River, future research on *C. calderoni* should be designed to (1) find still unknown localities and to (2) elaborate detailed distribution maps in order to (3) discover new conservation opportunities for this threatened Iberian endemic species. As many authors claimed (Rosenfeld and Hatfield, 2006) and performed for other cobitids (e.g., Przybylski and Valladolid, 2000; Oliva-Paterna *et al.*, 2002; Bohlen, 2003; Copp and Vilizzi, 2004), future research on life-history characteristics such as growth, survival, reproduction, fecundity, microhabitat requirements, etc., throughout the ontogeny of *C. calderoni* ought to be promoted as a necessary tool for this species management and conservation programmes.

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